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THE CONSTANCY OF CERTAIN PHYSIOLOGICAL CHARACTERS IN THE CLASSIFICATION OF BACTERIA.

H. A. HARDING

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THE CONSTANCY OF CERTAIN PHYSIOLOGICAL CHARACTERS IN THE CLASSIFICATION OF BACTERIA.*

H. A. HARDING.

SUMMARY.

1. During the past fifteen years constant effort has been made to find a workable system of classifying bacteria. The Classification Card of the Society of American Bacteriologists is the direct result of this effort.

2. The group number on this card is a numerical expression for the result of ten physiological reactions. Its value as a basis for classification depends upon the constancy with which the same numerical result is obtained from tests of various strains of a single species. When tested upon forty-four strains of Pseudomonas campestris (Pam.) Smith, the same group number, 211.3332513, was obtained for each strain.

3. The limitation of the group number system of classification lies in the fact that, as constituted at present, it probably does not carry the separation to a group synonymous with the ordinary conception of species. These results indicate that further assistance in classification may be expected from pathogenicity toward plants, indol formation, casein digestion, growth in Uschinsky's and Cohn's solutions and turbidity in broth. The technique of these determinations must be given further study before these reactions will be serviceable.

*Also presented at as a thesis before the Faculty of Cornell University for the degree of Doctor of Philosophy.

INTRODUCTION.

Science is commonly defined as an orderly arrangement of facts, and in practically all branches of biology a classification of species is the basis on which the facts are arranged. Bacteriology, if it can be said to have attained the dignity of a science, has thus far developed so primitive a plan of classification that the observed facts are in many cases in a condition little short of chaos.

The idea of species was originally based on morphological similarity combined with ability to produce fertile offspring by sexual reproduction. With bacteria sexual reproduction is unknown and morphology is so simple that it has barely sufficed to differentiate the genera. The early work with bacteria was largely confined to pathogenic organisms and pathogenicity was relied upon to define the limits of the species. As the study extended to non-pathogenic forms reliance was placed on various other physiological reactions, singly or in combination, but there has been little agreement among workers as to the relative value of the various reactions which were commonly recorded.

The difficulty of arriving at a conception of bacterial species, sufficiently clear-cut to be useful in classification, led to the recognition of groups of related species, of which the colon group was perhaps the earliest generally accepted example. These groups were to be treated as units until such time as the progress of knowledge would allow them to be broken up into their component species. This tendency to recognize groups rather than species was largely followed by Kruse[1] in the third edition of "Die Mikroorganismen," by Conn[2] in his "Classification of Dairy Bacteria," and by Chester[3] in his "Determinative Bacteriology."

As has been suggested, there is a lack of agreement among

[1] Kruse, W. Einleitende Bemerkungen zur Klassifikation. Die Mikroorganismen. Flügge. 3d edition. Vol. II, pp. 67–96. 1896.

[2] Conn, H. W. Classification of dairy bacteria. Storrs Agr. Exp. Sta., An. Rep. **12** (1899): 13–68. 1900.

[3] Chester, F. D. A manual of determinative bacteriology. 1901.

workers both as to the reactions which should be used in separating species and as to the relative importance of these reactions. Recognizing the importance of a uniform and concise method of recording such reactions and desiring to designate the more important among them, the Society of American Bacteriologists adopted an official classification card for this purpose. An important part of this card was the "group number," in which the results of ten different reactions were expressed numerically. This number was so designated since at first it was short and was intended to characterize a group of species. It has now been extended much beyond its original length.

Since a collection of classification cards is ordinarily arranged on the basis of the group number it is important to consider how far this group number can be used for this purpose without separating two strains of the same species. The constancy of the reactions which govern the group number is the vital point upon which the Society card must be judged as a standard for classification.

Until the first report to the American Public Health Association of its committee on media there was no satisfactory basis for computing the constancy of characteristics since it was not clear whether the variations which were observed were due to variations in the germs themselves or in the conditions under which the tests were made. The original selection of the group numbers was made on the basis of general impressions among bacteriologists aided by the work of Fuller and Johnson[4] and that of Gage.[5]

With the recognition of the usefulness of the group number there is a growing desire to extend the range of the number until it shall classify cultures as closely in accord with the idea of species as possible. Accordingly it is imperatively necessary to know both the constancy of these reactions and the extent to which the group number can be followed in classification without separating various strains of the same species.

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[4] Fuller, Geo. W. and Johnson, Geo. A. On the differentiation and classification of water bacteria. *Jour. of Exp. Med.* **4**: 609–626. 1899. Also in Amer. Pub. Health Asso. Proc. **25**: 580–586. 1899.

[5] Gage, S. DeM. Mass. Bd. of Health, An. Rep. 33 and following.

For this study it seemed desirable to select an organism in which the limits of what we commonly regard as a species are clearly defined and to study a large number of strains under as wide a variation in conditions as could be reasonably expected to occur in ordinary laboratory work. In doing this it was clearly recognized that the results which might be obtained with one species would not necessarily hold for all, but in view of the importance of this inquiry there seemed no better way than to make a beginning hoping that the example might stimulate others to extend the study.

The present study has been limited to various strains of one of the type species given on the society card, *Pseudomonas campestris* (Pam.) Smith, a form pathogenic to practically all of the cultivated *Cruciferae,* and one in which the culture characteristics are well known. Cultures were isolated directly from diseased plants as well as obtained through the courtesy of colleagues in different parts of the country. Thus the study included cultures which were so fresh as to have experienced the minimum effect of artificial cultivation in the laboratory as well as those which had been exposed to the vicissitudes of artificial media in different laboratories for many months. Most of these latter cultures passed through the prescribed course of revivification before being studied but in a number of cases this revivification was intentionally omitted. The study extended over a year and a half, using media prepared by different workers and in some cases the observations were made by three workers separately. In short, the effort was made to find the maximum variation which could be expected where observations were made in accordance with the official directions or with the deviation therefrom which could be reasonably expected in practice.

ACKNOWLEDGEMENTS.

This study has been conducted under the immediate direction of Prof. B. M. Duggar, and it is a pleasure to record the value of the suggestions received both from him and from Dean V. A.

Moore. Cultures were kindly furnished by colleagues in various laboratories as noted in the body of the report and much assistance was rendered by Messrs. M. J. Prucha and J. K. Wilson. In all cases where there seemed room for a difference of opinion as to the manner of recording the reaction of a culture they kindly rendered independent judgments on the point in question and in so doing added much to the value of the results here presented.

The quickness with which the details of even important events are lost is shown by the difficulty in assembling the facts regarding the origin of the classification card. The information on this point was largely furnished by Messrs. S. DeM. Gage, Erwin F. Smith, F. P. Gorham and C. E. Marshall.

To the friends who are here mentioned and to the larger additional number who have contributed in various ways to the success of this work sincere thanks are respectfully rendered.

SEPARATION OF BACTERIA INTO GENERA BASED ON MORPHOLOGY.

Many classifications of bacteria have been proposed by different authors. In the decade preceding 1900 it was customary to recognize three bacterial genera — Coccus, Bacillus and Spirillum — based on a morphological resemblance to the sphere, the rod and the spiral.

The described forms having become too numerous to be conveniently grouped under three genera, Migula,[6] in 1894, proposed using the arrangement of the flagella as a basis for increasing the genera. Since the appearance of the second volume of his "System der Bakterien"[7] in 1900, his classification has been generally adopted. While there has never been complete agreement on the question of the basis for erecting genera the dissenting workers have quite uniformly based their genera on morphology, so that in the past morphology has generally furnished the basis for the separation of bacterial genera.

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[6] Migula, W. Ueber ein neues System der Bakterien. Arbeiten aus dem bakt. Institut der Technischen Hochschule zu Karlsruhe. Bd. I. Heft I. 1894. Quoted from Migula. System der Bakterien. Bd. I, p. 46.

[7] Migula, W. System der Bakterien. Bd. II. 1900.

SEPARATION OF SPECIES ON A PHYSIOLOGICAL BASIS.

With the exception of spore formation, the morphological characters which are sufficiently definite to be useful in classification have been utilized by Migula in defining the genera so that the separation of species is necessarily on the basis of physiological reactions.

In his "System der Bakterien" Migula used as his main basis for separating the groups of species the chromogenesis, liquefaction, relation to air and form of colony growth in gelatin; the formation of spores and the manner of their germination; and in some cases the production of phosphorescence. With these reactions he divided the genera into groups and the species in each group were separated on whatever basis seemed most serviceable.

Chester[8] also followed the same general plan of dividing the genera into groups but he somewhat changed the basis on which these groups were formed. He retained the formation of spores as diagnostic but did not make use of the manner of their germination as Migula had done. He retained the chromogenesis, liquefaction and form of colony in gelatin, but used the terms aerobe and anaerobe instead of basing the division on the growth in gelatin stab.

To the reactions used by Migula for separating groups, Chester added Gram's stain, coagulation of milk, gas formation from dextrose and lactose and the nitro-indol reaction as well as the swelling of the rod in spore formation.

The classification of dairy bacteria by Conn[9] proceeded along the same general line, the aim being to reduce the flora of milk and its products to small groups which in many cases coincided with the common conception of species. He differed from Chester in not considering Gram's stain of sufficient diagnostic value to be used in this connection. He also made but slight use of the

[8] See footnote 3.

[9] Conn, H. W., Esten, W. M. and Stocking, W. A. A classification of dairy bacteria. Storrs Agr. Exp. Sta. An. Rep. **18** (1906): 91–203. 1907.

different relations of bacteria to oxygen although this had been emphasized by both Migula and Chester. On the other hand he considered the formation of acid from dextrose as of great importance, placing it second to that of liquefaction of gelatin, although neither Migula nor Chester had made any particular use of this reaction.

A critical inspection of the work of each of these authors brings out the fact that they have formulated a classification which concerned itself with the forms which had already been described and which made no particular provision for any forms which might be found at a later date. This is shown by the fact that in the classification within the various genera, even where the same reactions were used to separate the various groups, these reactions were not placed in the same sequence. The reason for this lack of uniformity was the desire of the authors to keep the final groups so arranged that those which were arbitrarily considered to be closely related should not be widely separated by the plan of classification. In other words, the authors had more faith in their general sense of relationship than in their ability to arrange the diagnostic reactions which they had selected in a logical manner. It is evident that any such makeshift could be of only temporary utility, since the rapidly increasing volume of new species would soon call for a rearrangement.

As has been indicated, each of these classifications was the result of a study of a large number of described forms, but each lacked an adequate provision for the placing of any new form which might be later encountered.

NEED OF A CONSTRUCTIVE CLASSIFICATION.

The fact is gradually coming to be recognized that if bacteriology is to take its place as a modern science the first requisite is a knowledge of the normal flora, including a knowledge of the effects upon that flora of the ordinary changes in its environment. Such a knowledge is fundamental to a proper understanding of diseases and fermentations and must be obtained before we can expect to control successfully the action of micro-organisms.

The obstacle which has thus far prevented any marked progress in this direction is the crudeness of our system of classification. The process of comparing an unknown form with the descriptions of previously studied organisms is extremely laborious, because practically every organism has been described on special media and under special conditions which must be duplicated before a satisfactory comparison can be made. Too often the conditions under which the earlier form was studied are unknown and an exact comparison is accordingly impossible.

This inability to use the results of previous workers has compelled each student to begin with the fundamentals of his problem, and the total progress in any line was practically limited to the product of a single individual since the laboriously acquired facts applied only to the circumstances under which they were observed. The absence of a common basis for comparing these isolated facts has prevented their orderly arrangement and has made accurate generalizations impossible.

The prime requisite for acquiring a knowledge of the bacterial flora is a classification which shall be so concise as to permit of fairly rapid progress in actual classification and yet so exact as to furnish a firm foundation upon which later workers can build. The aim of the first student of any field should be to reduce the flora to its main subdivisions, after which these subdivisions can be subjected to detailed study. Our knowledge of the action of bacterial protoplasm is yet so limited that no one can predict all of the reactions which will be ultimately used in classifying bacteria, but a considerable number are now known which will undoubtedly be useful in this connection.

One of the stumbling blocks in the past has been the attempt to produce a so-called natural classification,[10] that is, one which should record the steps by which the originally simple forms gradually evolved into those which were more complex. In bacteria we have the simplest form of life in which the protoplasm is

[10] A recent example is the system of O. Jensen. Die Hauptlinien des natürlichen Bakterien-systems. *Cent. f. Bakt.* **II, 22**: 305–346. 1909.

constantly responding to the stimuli of its environment and becoming modified in new ways. Considered chronologically, the differentiations of spore formation, enzym production and ability to live without a supply of free oxygen undoubtedly occurred in some definite order. Whatever this order may have been, the original forms have long since disappeared and their descendants have continued to differentiate until the forms which are now encountered present a bewildering complex of modifications.

After some workable basis of observation has been established and a large number of reliable and comparable observations are made available it may be possible to trace in part the order of original development. Until such data are available attempts at formulating such a natural classification are guesswork and are not worthy of serious scientific consideration. In the present state of knowledge it makes little difference what are taken as primary and as secondary lines of demarcation in any classification so long as the selected reactions really separate forms, are readily determinable and give constant and clear-cut results. On the other hand the applicability of a system of classification is increased by using only a limited number of differentiating reactions and using them constantly in the same sequence.

Two points in this connection stand out quite clearly. First, there is a growing tendency to use items which have the sharpness of chemical reactions, such as the production of gas and acid from sugar, instead of items which must be measured by general sense impressions, such as the form of colonies. Second, that in matters of classification the qualitative rather than the quantitative action is to be considered. The time will come when the first general arrangement of the flora is completed and the task of working up the groups in detail is begun when the influence of environment will be brought into strong relief by a study of the quantitative reactions. The main reason for not undertaking this study at the present time is the fact that the increase in routine which it entails would practically block progress.

ORIGIN OF THE SOCIETY CLASSIFICATION CARD.

The two features which most strongly commend the classification card are that it is in the form of a card and that the principal results are expressed numerically. The utility of cards for recording information in a readily accessible form was undoubtedly first brought prominently to the attention of scientists through the card catalogues at libraries.

The original suggestion of the use of numbers in connection with bacterial classification seems to have been in 1895 by Wyatt Johnson,[11] who said: "It has occurred to me that all of the important characteristics of a given species might be recorded more compactly than at present if a system were adopted by which the information furnished by the various tests could be represented by means of numbers, each stated in a definite order so as to form a code." The present application of these ideas has come in successive steps, each preparing the way for the succeeding one, and each logically following from what had preceded.

On the initiative of Dr. Wyatt Johnson of Montreal a convention of American bacteriologists was called by a committee of the American Public Health Association. This convention assembled in New York in June, 1895, discussed the situation carefully, and appointed a committee to draw up procedures for the study of bacteria in a uniform manner and with special reference to the differentiation of species. A statement in the report of this committee[12] to the American Public Health Association in 1897 well summarizes the situation. "The committee recognizes fully that these recommendations must of necessity be provisional. It publishes them in the hope that by this act it will direct attention to the urgent need now existing for a full and accurate

[11] Johnson, Wyatt. On grouping water bacteria. Amer. Pub. Health Asso. Proc. **20**: 445–449. 1895.

[12] Proceedures recommended for the study of bacteria with especial reference to greater uniformity in the description and differentiation of species. Being the report of a committee of bacteriologists to the Committee on the Pollution of Water Supplies of the Amer. Pub. Health Association. Amer. Pub. Health. Asso. Proc. **23**: 60–100. 1898.

description of species of bacteria in which the items have been determined by methods common to the main body of workers, and as a consequence are capable of verification and control." The activities of this committee, which rendered a final report in 1904,[13] by unifying methods of making media and observing cultures, furnished an indispensable foundation for the accumulation of a mass of comparable facts regarding the characteristics of bacteria.

Given this constantly accumulating mass of somewhat comparable observations, the next question was the selection of those reactions which were sufficiently constant with any given species to warrant their being used to characterize the species. While this question is yet far from being settled in its entirely, a good beginning was made by Fuller and Johnson[14] in 1899. With seven different species which had been long cultivated in the laboratory they obtained diametrically opposed results when tested before and after a preliminary cultivation to return them to a vigorous condition. After such preliminary cultivation, eleven species from water gave 100 per ct. of constancy with each of fourteen selected reactions. These authors divided the water bacteria which they had studied into thirteen groups on the basis of fluorescence, chromogenesis, liquefaction, form of colony and fermentation of carbohydrates. They separated the members of each group on the basis of twenty-six reactions, the behavior of each culture being expressed by + or —. Using this manner of recording their observations they presented the classification and culture reactions of forty-two species on a single chart, a striking illustration of clearness and conciseness.

This manner of presenting results was at once taken advantage of by Conn.[15] As his adaptation was made shortly before the publication of the results from a long study of dairy bacteria,

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[13] Report of Committee on Standard Methods of Water Analysis to the Laboratory Section of the Am. Pub. Health Asso. *Jour. of Inf. Dis.* Sup. No. I, May, 1905.

[14] See footnote 4.

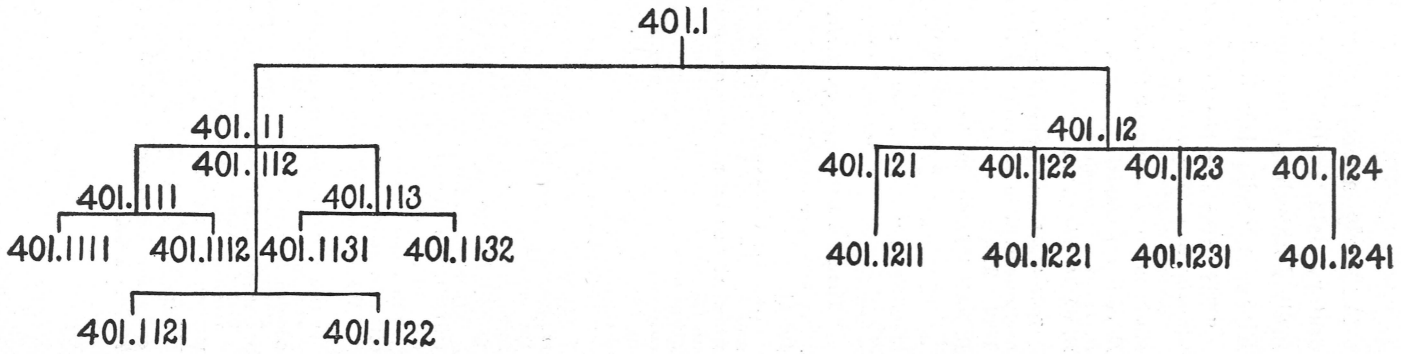
[15] See footnote 2.

some modifications of the form were necessary. He says: "For these reasons the tables which I have been obliged to make out and to use differ in some details from those of Fuller and Johnson. I have, however, followed them as closely as possible." The dairy flora was divided into ten groups instead of thirteen, the first four being identical with the fluorescent and chromogenic groups of Fuller and Johnson, the remainder being formed on the basis of rod or coccus form, liquefaction and spore formation. These groups were subdivided on the basis of thirty-three headings which differed but slightly from those used by Fuller and Johnson. In addition to this table, Conn gave a written description of each species which extended and explained the tabular results. The arrangement of the headings employed by Conn will be better understood by referring to the accompanying reproduction of Conn's card. Fig. 1. This list omits liquefaction of blood serum, nitrate and indol production and pathogenicity toward mice as used by Fuller and Johnson and adds the columns headed coccus, uniting in chains, sediment, proteus-like, moss-like, deep-funnel, needle growth, surface growth, gas produced, acid, alkaline and slimy.

FIG. 1.— CONN'S FIRST CARD.

Lab. No.	NAME.	MORPHOLOGY.						CULTURAL FEATURES																BIOCHEMICAL FEATURES.													
								Nutrient broth tube.			Nutrient agar tube.			Gelatin plate.			Gelatin stab.				Potato tube.																
		Bacillus.	Coccus.	Diam. greater than 1μ.	Motile.	Uniting in chains.	Spores.	Turbidity.	Scum.	Sediment.	Dull.	Wrinkled.	Characteristic.	Proteus-like.	Moss-like.	Liquefies.	Deep funnel.	Needle growth.	Surface g'wth.	Gas produced	Visible	Luxuriant.	Fermentation tube Growth in closed arms.	Grows at body temperature.	Facultative anaerobic.	Milk.											
																										Curdles.	Acid.	Alkaline.	Digests.	Slimy.	Strong odor.	Chromogenesis.	Fluoresence.	Gas production.	

FIG. 2.— DIAGRAM ILLUSTRATING RICKARD'S SYSTEM.



An early attempt at using cards for recording general laboratory data, including a numerical system for designating the individual cultures from which the data were derived was made by Rickards,[16] who described it as follows:

"The writer's system is an adaptation of the Dewey Decimal System of Classification,* the method of use being such as is easily remembered.

"Every species of bacteria, upon becoming a member of the laboratory stock, is given a number in the hundreds. Thus:

B. coli communis	100	*B. mallei*	400
B. typhi abdominalis	200	*B. prodigiosus*	500
B. diphtheriae	300	*B. pestis bubonicae*	600

Individual specimens of any one species; coming from different sources, are numbered in the order of their isolation or reception with the units from 1–49. Thus:

B. mallei from	one horse	401
" " "	a second horse	402
" " "	a different lesion in the second horse	403
" " "	same lesion at a different time	404
" " "	a third horse	405

"The first culture of *B. mallei* isolated would be 401.1.

"A sub- (or daughter) * * culture from this original culture would be 401.11.

"A sub- (or daughter) culture from this second culture would be 401.111, and so on — each *sub*-culture bearing the number of the mother culture from which it was taken, with one figure more placed one space more to the right of the decimal point. If but one sub-culture is made this added figure is always one. If more than one sub-culture is made the first of these sister cultures is designated as above (the number of the mother culture with one in the next right decimal place), the second by two in the same place of decimals, etc., 401.11, 401.12, 401.13, etc.

"This may be better illustrated by a graphical sketch [Fig. 2.]

(It will be seen from the above that a single daughter culture is always expressed by the number of the mother culture with the figure 1 placed in the next right place of decimals, and that further cultures made from the same given mother culture are expressed by increasing this last new figure in arithmetical order. This is the key to the system.)

"If at any time more than nine sister cultures are made from any one culture, the figures above nine are inclosed in brackets to avoid confusion, *e.g.*, 404.18, 404.19, 404.1(10), 404.1(11), etc.

"In cases where the numbers have become somewhat unwieldy, they may often be abbreviated by using exponents, *e. g.*, $401.1111121113 = 401.1^{5}21^{3}3$.

"When an *unidentified* organism is *isolated*, it is given the specific num-

* Dewey, M. Decimal classification and relativ index.

** "For the sake of convenience and clearness, the following terms have been adopted in this article:

Mother culture.— The culture from which another culture is inoculated.

Daughter culture.— The sub-culture from the mother culture.

Sister cultures.— Two or more cultures made from the same mother culture.

(Obviously the terms are relative. It is evident that any one culture may be a mother, a daughter, and a sister culture at the same time.)"

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[16] Rickards, B. R. A system of recording cultures of bacteria genealogically for laboratory purposes. Boston Health Dept. An. Rep. **30** (1901): 75–79. 1902.

ber in hundreds which designates the species which it most resembles, but with the tens and unit figures running above 50; thus, a glanders-like organism would be numbered 451, etc., pending its further examination. If found to be glanders it would be renumbered below 450, taking the number next above that of the glanders culture last isolated.

"*Unidentified* organisms having no striking resemblance to any species possessed by the laboratory are classed by themselves under one species number (*e. g.*, 10,000), until identified.

"A card system is used in connection with this system of numbering, offering a complete record."

The next marked advance in the matter of keeping records with a view to ease of comparison and classification of results was made at the Lawrence Experiment Station[17] in connection with the routine examination of water, principally for *B. coli.* This contribution was noteworthy because it introduced the numerical expression for the group (group number) and emphasized the card as the best form for keeping and comparing such records. It is further important because some of the suggestions in it might be applied with profit to our present official classification card.

It can be best described in the words of its authors:[18]

"The attempt at a numerical classification at the Experiment Station arose in an effort to classify existing bacterial literature under the decimal system in common use for library cataloging. The credit for the first practical suggestion along this line should be given to A. I. Kendall, at that time a member of the bacteriological force at the Experiment Station, from whose preliminary scheme the system in use at the present time has been the logical outgrowth. The system of numerical classification has already been described by one of the writers,* the extension of this system to the complete record, with the use of cards, is here described for the first time.

"In the system all of the characteristics of a species are described by number, this number being derived from the combination generally of two or more allied characteristics.

"The group number is represented by four figures, of which the first two digits signify the morphological genera, the form, method of division, motility, and arrangement of flagella, according to the accepted classification of Migula.

These are derived as follows:

1. *Coccaceae.*
   11. Streptococcus, division in one plane.
   12. Micrococcus, division on two planes.

* [See footnote (17).]

[17] Gage, S. DeM. Bacteriological studies at the Lawrence Experiment Station with special reference to the determination of *B. Coli.* Mass. Bd. of Health. An. Rep. **33** (1901): 397–420. 1902.

[18] Gage, S. DeM. and Phelps, E. B. On the classification and identification of bacteria with a description of the card system in use at the Lawrence Experiment Station for records of species. Amer. Pub. Health. Asso. Proc. **28** (1902): 494–505. 1903.

13. Sarcina, division in three planes.
14. Planococcus, motile coccus.
15. Planosarcina, motile sarcina.

2. *Bacteriaceae.*
20.**Motile rods, flagella not determined.
21. Bacterium, non-motile rods.
22. Pseudomonas, *a*, motile rods, flagella monotrichic.
23. Pseudomonas, *b*, motile rods, flagella lophotrichic.
24. Bacillus, motile rods, flagella peritrichic.

3. *Spirillaceae.*
31. Spirosoma, cells rigid, without flagella.
32. Microspira, cells rigid, one (rarely 2–3) polar flagellum.
33. Spirillum, cells rigid, polar flagella tufts.
34. Spirochaeta, cells flexuous.

The morphological genera are sub-divided by the differences in certain well-recognized cultural and biochemical features.

This sub-division, forming part of the whole group number, consists of two digits, of which the third group figure indicates the biochemical features of the liquefaction of gelatin and the production of gas or acid in dextrose broth, as suggested by Groups IX to XIII of Fuller and Johnson. The derivation of the nine digits used in this place is as follows:

	Gelatin.	*Dextrose Broth.*
1.	Non-liquefied.	No gas or acid produced.
2.	Non-liquefied.	Acid produced, no gas.
3.	Non-liquefied.	Gas produced.
4.	Liquefied.	No gas or acid produced
5.	Liquefied.	Acid produced, no gas.
6.	Liquefied.	Gas produced.
7.	†Doubtful.	No gas or acid produced.
8.	†Doubtful.	Acid produced, no gas.
9.	†Doubtful.	Gas produced.

"The digit in the fourth place shows the fluorescence and chromogenesis on agar, the grouping being similar to that in Groups I to VIII of Fuller and Johnson.

"These are distinguished as follows:
1. All fluorescent species, irrespective of their chromogenesis.
2. All red chromogenic species.
3. All orange chromogenic species.
4. All yellow chromogenic species.
5. All blue and violet chromogenic species.
6. All green chromogenic species.
7. All brown chromogenic species.
8.
9. All others not included in the above.

"By the use of such a system as the above, the arrangement of bacteria into groups becomes at once a simple and exact matter. We no longer have the old, rather indefinite question, does our species belong to the colon

** 20 is provisional group, and should disappear, its members falling into groups 22, 23 or 24.

† In groups 7, 8 and 9, would be included the so-called thermophylic species which do not grow at the temperature at which gelatin is solid.

group, or to the hog cholera group, or to some other equally indefinite group? We simply drop our colon bacillus into slot No. 2439, or our sewage stroptococcus into No. 1129, and they immediately find themselves in congenial company.

"The system of recording reactions by means of plus or minus signs, used by Fuller and Johnson in their compilation of the bacterial flora of the Ohio river, was a distinct advance over the verbose written descriptions of earlier investigators.

"To reduce the size of the tables and render comparisons more easy we may either reduce the number of tests tabulated or we may combine these tests in some manner so that two or more tests may be recorded in the space at present occupied by one.

"The reduction of the number of tests is not feasible if we would wish to have anything like complete data. As a means for combining the data without curtailing either its volume or its usefulness, the numerical system here described has proved quite successful.

"As a basis for the system we start with the assumption that all of the tests usually applied in species differentiation may be logically divided into groups of three, any one, two or three of which may be positive. Arranging these three tests, A, B, C, in the order of negatives we derive eight numbers, each one representing some combination of the positive and negative values of the three tests, as follows:

	A	B	C	
1	0	0	0	All negative.
2	0	0	+	
3	0	+	0	Two negative, one positive.
4	+	0	0	
5	0	+	+	
6	+	0	+	One negative, two positive.
7	+	+	0	
8	+	+	+	All positive.

"A glance at any one of the tabular descriptions of species of the present day will show that there are usually three characteristics recorded on each medium, and an arrangement by media is at once suggested. There are some tests, however, which at first appear to introduce complications. For example, in the arrangement of tests further on, under milk, we have coagulation, acid reaction, and alkaline reaction as values for A, B, C in the table of numbers. It is manifestly impossible for a reaction to be at the same time acid and alkaline.

"This, however, does not vitiate the values for the other numbers, the digits 5 and 8 simply becoming non-existent. Under aerobiosis we have a more extreme case, only one of the three values used being possible at one time. In this case all of the digits except 2, 3 and 4 become silent, but as we are concerned only with the live numbers, this tends to simplify, rather than to complicate matters. In the case of pathogenesis and the indol

FIG. 3.— TABLE SHOWING GROUPS OF TESTS UNDER NUMERICAL SYSTEM.

Column	GROUP.	A.	B.	C.	Digits.
1	Morphology	Spores	Capsules	Gram stain	1 to 8 inclusive.
2	Liquefaction	Gelatin	Casein	Serum	1 to 8 inclusive.
3	Dextrose broth	Gas	Acid	Growth in closed arm	1 to 8 inclusive.
4	Lactose broth	Gas	Acid	Growth in closed arm	1 to 8 inclusive.
5	Saccharose broth	Gas	Acid	Growth in closed arm	1 to 8 inclusive.
6	Milk	Coagulated	Acid	Alkaline	1, 2, 3, 4, 6, 7 only.
7	Nitrate reduced	Nitrites	Ammonia	Nitrogen	1 to 8 inclusive.
8	Indol produced				1 and 8 only.
9	Nutrient broth	Turbid	Pellicle	Sediment	1 to 8 inclusive.
10	Gelatin stab	Craterform	Infundibuliform *	Stratiform	1, 2, 3, 4 only.
11	Agar streak	Luxurient	Dull or winkled	Viscous	1 to 8 inclusive.
12	Potato	Visible	Luxuriant	Discolored	1, 2, 4, 6, 7, 8 only.
13	Temperature	Grows better at 20° C.	Grows equally well at 20° or 38°	Grows better at 38° C	1, 2, 3, 4 only.
14	Aerobiosis	Obligate aerobe	Facultative	Obligate anaerobe	2, 3, 4 only.
15	Pathogenesis				1 and 8 only.

* Including napiform and saccate.

reaction, where we have the positive and negative values for only one function, we should use only the digits 1 and 8 as representing the extreme negative and positive limits.

"Of course it would be possible to have a separate arrangement for such cases as these, or we might combine two or more of them, but this would tend rather to confusion than to simplicity. Throughout the system as arranged, only similar tests have been combined, the application of a number of other combinations having been shown by a trial to lead to confusion.

"In the application of the system to various tests, only such tests have been retained as have given constant results, and which can be duplicated at any time with media of a constant composition and with cultures in the proper condition for study, *i. e.*, after efficient preliminary cultivation. The order of the tests is based on their probable value in species differentiation. This order, with the division of the tests into groups, and the various figures representing the various combinations which would appear in a tabulation of species by this method, is shown in the preceding table. (Fig. 3.)

"The writers have devised a form for use at the Experiment Station, which proves to be so convenient, that it is here described for the benefit of others who wish to break away from the inconveniences of the older methods of keeping species records. The form shown is self-explanatory. Records are made in the columns on the right by plus and minus signs and a few well-known symbols. There is sufficient room in the body of the blank for notes and for such descriptive matter as is not recorded in the columns, while additional notes and drawings may be made at the bottom and on the back of the card.

"The entries in the spaces across the top are made by the numerical system heretofore described. The form is printed on a good grade of card stock, this being easier to handle and more durable than paper. The size of the card, eight by ten and one-half inches, is the same as the regular letter sheet, and cards may be filed in any of the numerous vertical cabinets which are on the market. The cards are filed in numerical order by the system already described. By this method, similar species come on adjacent cards and it is a simple matter to weed out identical cultures. Comparisons of descriptions are made by laying the cards down so that only the marginal columns are visible, the comparison being either the plus and minus signs or the numerical nomenclature, the numerical grouping rendering the comparison of only a few cards necessary at any one time. We have found it to be very convenient to enter descriptions of other writers on this form, filing these cards with our own descriptions, thus eliminating reading back and forth from one system to another. The reduction of other descriptions to this form is not as laborious as would at first appear, the ruling of the form being so spaced that the copying may be readily done on the typewriter.

"The ruled form follows: [Fig. 4.]

[FIG. 4.—] FORM USED FOR BACTERIAL DESCRIPTIONS AT THE LAWRENCE EXPERIMENT STATION.
(Reduced.)

Group.	Morph	Liq.	Dex.	Lact.	Sac.	Milk	KNO_3	Indol.	Broth.	Gel.	Agar.	Potato.	Temp.	Aerobe.	Path.		Culture.
NAME.						INVESTIGATOR.					SOURCE.				ISOLATED.		

	Morphology.	Spores	Length	Diameter	Form
		Capsules	Gram's	Flagella	Motile
	Gelatin Stab.			Form	Liq.
	Gelatin Plate.			Form	Liq.
Dextrose.	Fermentation Tube.		C. A.	Acid	Gas
Lactose.			C. A.	Acid	Gas
Saccharose.			C. A.	Acid	Gas
	Agar Streak.	Lux.	Viscous	Wrinkled	Dull
				Chrom.	Fluor.
	Agar Plate.				
	Broth.		Sediment	Pellicle	Turbid
	Milk.		Reaction	Pept.	Coag.
	Nitrates.		N.	NH_3	HNO_2
	Indol.				
	Potato.		Discolor	Lux.	Visible
	Serum.				Liq.
	Pathogenesis.				
	Litmus Lactose Agar.			T. B.	Red
	Temperature.			38°C.	20°C.
	Odor.				

This work by Gage and his associates was in many ways the most important contribution which has been made to this plan of classification, since it introduced not only the use of the card and the group number but also many other details which have been or will be used in this connection.

At the same meeting of the American Public Health Association, Kendall,[20] who had assisted in the development of the Lawrence card while an assistant to Gage, presented a similar card with the additional feature that the details of the culture growths were expressed by numbers. When the time comes for the study of the effect of changes in environment upon the finer details of culture characteristics the plan of Kendall or something similar will be found useful. At the present stage of the science his system is entirely too cumbersome.

Conn now prepared a new card which was a copy of that of Gage with some extensions, particularly in the matter of milk. This was natural, as Conn was principally interested in the milk flora. A copy of this card is given in Fig. 5.

During his extended study of bacteriological literature Chester[21] had been impressed with the futility of the current methods of describing species and was looking for an improved method. The paper by Gage and Phelps before the American Public Health Association impressed him so strongly that in August, 1903, he applied to Gage[22] and obtained a supply of the Lawrence cards for use in his own laboratory.

The failure of the American Public Health Association committee to proceed after its report on standard media created a general feeling that the Society of American Bacteriologists should take up the problem of classification. Chester presented the matter so forcibly at the Philadelphia meeting in December, 1903, that a Committee on Identification of Bacterial Species was

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[20] Kendall, A. I. A proposed classification and method of graphical tabulation of the characters of bacteria. Amer. Pub. Health Asso. Proc. **28** (1902): 481–493. 1903.

[21] See footnote 3.

[22] Letter to S. DeM. Gage, dated Aug. 20, 1903.

appointed consisting of Messrs. F. D. Chester, F. P. Gorham and Erwin F. Smith.

At the Philadelphia meeting in December, 1904, Chester presented a paper[23] in which he introduced and explained the group number as given on page 25. This paper was virtually a preliminary report of his committee, presented for suggestions and criticism. In the autumn of 1905, the first society card, reproduced in Figs. 6 and 7, and a separate explanatory folder, given below, were distributed to members of the society. This first card was 5 x 8 inches, printed on both sides.

THE SOCIETY OF AMERICAN BACTERIOLOGISTS.

Preliminary Report of the Committee on Methods for the Identification of Bacterial Species.

The study of bacteria for purposes of grouping calls for a recognition of two classes of characters, (1) salient features, which have primary value taxonomically and (2) detailed features, which serve to distinguish strains, races and varieties.

The division of the Schizomycetes into genera is that proposed by Migula and is based upon morphology. The division of the genera into groups is as yet a provisional, or a purely artificial one. It serves however to identify organisms, and is useful at least to that extent.

The salient features of an organism belonging to any one genus can be conveniently expressed by a series of digits, representing a whole number and a decimal. This system readily enables organisms having similar characters to be brought together and grouped about some central organism or type. The system is shown in Table I.

An attempt has been made to arrange the characters in the order of their importance. It will be observed that chromogenesis has been placed last. This is contrary to prior notions. Chromogenesis is a variable character dependent upon environment. Organisms frequently show constancy in all the characters which precede but may show altered or negative chromogenesis. Many forms are identical in all the preceding characters and differ among themselves only in the presence or absence of pigment. To separate them widely would do violence to a rational system of grouping.

The past literature of bacteriology abounds in such imperfect descriptions of organisms as to make their grouping, according to any system, impossible. This fact calls for the adoption of some scheme to which all descriptions shall conform, in order that no essential character shall be overlooked.

The accompanying card is proposed for the recording of the characters

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[23] Chester, F. D. Principles of classification of bacteria. Report of Phila. Meeting of Dec. 27–8, 1904. In *Science* N. S. **21**: 485–486. 1905; and *Cent. f. Bak.* **II, 15**: 240–241. 1905.

FIG. 5.— CONN'S SECOND CARD.

Group	Morph	Liq.	Tex.	Lact.	Sac.	Milk	KNO3	Indol	Broth	Gel. St.	Agar	Potato	Temp.	Gerahe	Path.	Culture

NAME | INVESTIGATOR. | SOURCE | ISOLATED.

Morphology		Spores	Length	Diametr	Form
		Capsuli's	Gram's	Flagella	Motile
Gelatin Stab		Begins	Complte	Form	Liq
Gelatin Colony		Mica Large	Mica Small	Form	Liq
Litmus Gelatin Colony			Acid	Form	Liq
Fermentation Tube Dextrose	3 Days Later		C. A	Acid	Gas
Lactose	3 Days Later		C. A	Acid	Gas
Saccharose	3 Days Later		C. A	Acid	Gas
Agar Streak		Lux	Viscus	Krinkld	Dull
				Chrom	Fluor
Temperature				20°	38°
Broth	3 Days Later		Sediment	Pellicle	Turbid
Milk	3 Days Later	Acid 20°	Acid 38°	Alk. 20°	Alk 38°
Curdling	3 Days Later	20°	38°	Dig 20°	Dig 38°
			Color	Odor	Slimy
Potato			Discolor	Lux	Visible
Indol					
Pathogenesis					
Litmus Lactose Agar				T.B	Red
Nitrates			NH_3	HNO_2	

FIG. 6.— FRONT OF FIRST SOCIETY CARD

SALIENT FEATURES (Partly included in Group No.)

Genus	Group No. or Character Complex[1]	Morph.			Cultural Features																						Biochemical Features										Additional Salient or Diagnostic Features
					Broth			Agar		Gel. Plate					Gel.Stab			Potato										Liquefaction[7]			Milk								
Name		Diam. over 1 micron.	Chains	Spores	Turbidity	Scum	Sediment	Dull	Wrinkled	Round, Compact	Proteus-like	Rhizoid	Filamentous	Curled	Funnel	Surface Growth	Needle Growth	Starch destroyed	Abundant	Discolored	Grows at 37°C	Gelatin	Casein	Blood Serum	Curdled	Acid	Alkaline	Indol	H_2S	Ammonia	Nitrates reduced	Gram's stain						
Source																																					
Cult. No.																																					

DETAILED FEATURES.

I. MORPHOLOGY.

1. Vegetative Cells.
   Form, *round, short rods, long rods, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate.*
   Limits of Size.[3]
   Ends, *rounded, truncate, concave.*
   Agar Block[2]:
   - Orientation (grouping).
   - Chains (No. of elements),[4] ..... *short chains, long chains.*
   - Orientation of Chains, *parallel, irregular.*

2. Sporangia.
   Form, *elliptical, short rods, spindled, clavate.*
   Limits of Size.
   Agar Block:
   - Orientation (grouping).
   - Chains (No. of elements).
   - Orientation of Chains.

   Location of Spores.

3. Spores.
   Form, *round, elliptical,* (2× diam.) *elongated.*
   Limits of Size.
   Wall.
   Naked.
   Sporangium wall adherent.[5]
   Germination, *equatorial, oblique, polar, bipolar, by stretching.*

4. Flagella No. .....
   Arrangement.

5. Capsules, present on .....

6. Staining Reactions.
   1:10 watery fuchsin .....
   Special Stains .....
   Fat .....
   Neisser .....
   Glycogen .....

II. CULTURAL FEATURES.[6]

1. Agar Stroke.
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effused, raised, convex.*
   Lustre, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Optical Characters, *opaque, opalescent.*
   Chromogenesis.
   Consistency, *slimy, butyrous, viscid, membranous, coriaceus, brittle.*
   Medium discolored.

2. Potato.
   Form of growth (as before).
   Elevation " "
   Lustre " "
   Chromogenesis.
   Consistency as before.
   Medium discolored.

3. Blood serum.
   Form of growth (as before).
   Elevation " "
   Lustre " "
   Topography " "
   Chromogenesis.
   Medium discolored.
   Liquefaction .....10d..... 4w.....

4. Agar Stab.
   Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*

5. Gelatin Stab.[7]
   Line of puncture (as before).
   Form of liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform.*
   Surface growth.

NOTE—Underscore required terms. Observe accompanying notes and glossary of terms.

FIG. 7.— BACK OF FIRST SOCIETY CARD.

6. Broth.
   Surface growth, *ring, scum, floculent, membranous, none.*
   Turbidity, *slight, moderate, strong, transient, persistent, none.*

7. Milk.
   Coagulation.
   Liquefaction .....10 d.....4 w.....
   Reaction in 2 d[8].....4 d.....10 d.....
   Consistency, *slimy, viscid, unchanged.*
   Medium discolored.
   Milk agar.

8. Gelatin Colonies.
   Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
   Elevation, *flat, effused, raised, convex, pulvinate, crateriform, (liquefying).*
   Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, cilliate, filamentous, flocose, curled.*
   Internal structure, *amorphous, finely—coarsely-granular, grumose, filamentous, flocose, curled.*

9. Agar colonies.
   Form (as before) .....
   Elevation " " .....
   Edge " " .....
   Internal Structure (as before) .....

10. Relative growth at 20° and 37°C.

III. BIOCHEMICAL FEATURES.

1. Fermentation of dextrose.
   Gas production.
   H. CO_2 ratio.
   Growth in closed arm.
   Acidifying coefficient [9]2 d....4 d....10 d.....

2. Fermentation of lactose.
   Gas production.....
   H. CO_2 ratio.
   Growth in closed arm.
   Acidifying coefficient 2 d....4 d.....10 d.....

3. Fermentation of saccharose.
   Gas production.
   H. CO_2 ratio.
   Growth in closed arm.
   Acidifying coefficient 2 d....4 d.....10 d.....

4. Fermentation of .....

5. Ammonia production,[10] *feeble, moderate, strong.*

6. Reduction of nitrates in nitrate broth.[11]
   Presence of nitrites.....
   " " ammonia.....
   " " nitrates.....
   " " free nitrogen.....

7. Indol.
8. H_2S.
9. Starch Jelly.

IV. PATHOGENESIS.

V. ADDITIONAL DATA.

of an organism. The salient features at the top of the card are expressed by the group number and by + or — signs.

The detailed features are expressed by means of an appropriate terminology.

The cards can be filed like catalogue cards, and arranged in accordance with the group number, thus bringing similar organisms together and rendering comparison easy. The reference numbers scattered through the text of the card refer to the appended notes. There is also attached a glossary of terms.

TABLE I.

A Numerical System of Recording the Salient Characters of an Organism.

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.0001	Nitrates reduced
.0002	Nitrates not reduced
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue chromogens
.00004	Green chromogens
.00005	Yellow chromogens
.00006	Orange chromogens
.00007	Red chromogens
.00008	Brown chromogens
.00009	chromogens
.00000	Non-chromogenic

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

BACILLUS COLI (Escherich) Migula becomes	B.	212.11110
BACILLUS ALCALIGENES Petruschky becomes	B.	212.33310
PSEUDOMONAS CAMPESTRIS (Pammel) Smith	Ps.	211.33315
BACTERIUM SUICIDA Migula (Pammel) Smith	Bact.	212.2320

NOTES.

(1) For decimal system of group numbers see Table I.

(2) Hill: "Hanging-block" Preparations for the Microscopic Observation of Developing Bacteria: Jour. Med. Research, 1902, I, No. 2. Chester: A Review of the Bacillus Subtilis Group of Bacteria, Centralblatt f. Bakteriol, 2 te Abt. 1904, XIII, 739.

(3) Chester: l. c. p. 738.

(4) Chester: l. c. p. 741.

(5) Chester: l. c. p. 742.

(6) The composition, method of preparation, and reaction of all media should be given in connection with the description of cultural features (See revised methods of Laboratory Section of Am. Pub. Health Assoc., 1905).

(7) Gelatin stab cultures should be held for 4 weeks to determine liquefaction.

(8) Remove an oese of the culture, deposit same on strip of Squibb's neutral litmus paper, by the side of which is placed a similar quantity of the blank, or titrate 5 c. c. with $\frac{N}{20}$ NaOH.

(9) Titrate with $\frac{N}{20}$ NaOH using phenolphthalein as an indicator: make titrations at same times from blank. Acidifying coefficient equals titre of culture divided by titre of blank.

The titration should be done after boiling to drive off any CO_2 present in the culture.

(10) Place 1 c. c. of culture in one 50 c. c. Nessler jar and 1 c. c. of blank in another; fill to 50 c. c. mark with ammonia-free water; add 2 c. c. of Nessler reagent to each tube and compare tints; compare tints before precipitation occurs.

(11) Nitrates may be reduced to ammonia and free nitrogen and no nitrates may be present: this, however, is equivalent to a reduction. Test for nitrites by the starch, iodide of potassium, sulphuric acid test. Determine nitrates in culture and blank by the phenol-sulphonic acid method, using 1 c. c. of each.

Also compare ammonia, by method in note 10, in nitrate and plain broth cultures of same age and also in uninoculated nitrate broth of same batch.

GENERAL NOTE — Observations on morphology of vegetative rods to be made on 18 to 24 hour agar stroke cultures grown under optimum conditions. Observations of cultural features to cover a period of at least 10 days. Determination of acid production to be made on second, fourth and tenth days of growth. Ammonia, indol and nitrite tests to be made at end of tenth day. Observations on liquefaction to be extended four weeks.

GLOSSARY OF TERMS.

ARBORESCENT, a branched, tree-like growth.

AMEBOID, assuming various shapes like an amœba.

BEADED, in agar strokes, disjointed or confluent colonies; in agar stab disjointed or confluent colonies along the line of inoculation.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS,

Short chains composed of 2–8 elements.

Long chains composed of more than 8 elements.

CILIATE, having fine hair-like extensions, like cilia.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flatly convex.

CORIACEOUS, growth tough, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

CURLED, composed of parallel chains in strands, as in anthrax colonies.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSED, growth thin, veily, usually spreading.

ENTINE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.
FILAMENTOUS, growth composed of long irregularly placed or interwoven filaments.
FILIFORM, in agar stroke a uniform streak along line of inoculation; in stab cultures a uniform growth along line of inoculation.
FIMBRIATE, border fringed with slender processes, larger than filaments.
FLOCOSE, growth composed of short curved chains, variously orientated.
INFUNDIBULIFORM, form of a funnel or inverted cone.
LACERATE, having the margin cut into irregular segments as if torn.
LOBATE, border deeply undulate, producing lobes (see undulate).
MEMBRANOUS, growth thin, coherent, like a membrane.
MYCELIOID, colonies having the radiately filamentous appearance of mould colonies.
NAPIFORM, liquefaction with the form of a turnip.
PLUMOSE, a fleecy or feathery growth.
PULVINATE, in the form of a cushion, decidedly convex.
PUNCTIFORM, very minute colonies, whose form cannot be seen with the naked eye.
RAISED, growth thick, with abrupt or terraced edges.
RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides.*
RUGOSE, wrinkled.
SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.
SPREADING, growth extending much beyond the line of inoculation.
UNDULATE, border waved, with shallow sinuses.
VERRUCOSE, growth wart-like, with wart-like prominences.
VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils, as in potato cultures of the potato bacillus.
VILLOUS, growth beset with hair-like extensions.
VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

For further terminology see Manual of Determinative Bacteriology, Chester. 1901, pp. 381–86.

This first card and accompanying explanation were almost exclusively the work of Chester, the other two members having made only a few suggestions.[24] While it contained little that was strictly new in principle, it was a decided improvement on what had preceded it. The first two places in the Lawrence group number were rendered superfluous by designating the genera according to the system of Migula, thus restricting the group number to the separation of species. The group number was extended to eight places but the supplementary numbers on the Lawrence card, which were virtually part of the group number, were eliminated. This latter action was not in the line of progress. The need of a method of tersely expressing those characteristics which

[24] Details of the committee action have been kindly supplied by Drs. Gorham and Smith.

are not accepted as being of classificatory rank is evident. The additions under the head of "Detailed Features" was an improvement, a step toward accommodating a complete record of an organism upon a single card.

The samples of this card and accompanying folder were distributed to the members some months before the Ann Arbor meeting in December, 1905, and were discussed at that meeting.

The principal objections raised against the first card were the evident inconvenience of consulting data on both sides of the card and the fact that the record of + and — was in the body of the card where it was not easy to make comparisons with other cards. Both of these objections were met in the second society card, which appeared during 1906, and is reproduced in Figs. 8 and 9.

This card was 8 x 10 inches, the increased size allowing all of the data to be placed on one side, the reverse side furnishing the information previously given by an accompanying folder. The arrangement of the headings was also improved, the spaces for the + and — signs being at the margin to facilitate the comparison of a number of cards.

The activities of Professor Chester in this connection practically ceased with this second card, as he went into commercial work and later severed his connection with the society.

When discussed by the society at the New York meeting in 1906, the desire was expressed to have a card sufficiently extensive to accommodate all of the data which should be necessary in describing a new species and at the same time have a provision for emphasizing the points most important in classification. The burden of fulfilling these instructions fell mainly upon Dr. Erwin F. Smith. The result was the 8½ x 10½-inch card adopted by the society at the Chicago meeting. This card is shown in Figs. 10 and 11, the reactions for *Ps. campestris* being indicated to make the form self-explanatory.

This third card, which was indorsed by the Society of American Bacteriologists December 31, 1907, differs from its predecessors in that the group number, brief characterization, detailed features

FIG. 8.— FRONT OF SECOND SOCIETY CARD.

Genus	Group No.[1]	Morphology[2]					Cultural Features[2]														Biochemical Features										Additional Salient or Diagnostic Features
		Diam. over 1 micron.	Chains	Endospores	Motility	Gram's Stain	Broth: Turbidity	Broth: Scum	Broth: Sediment	Agar: Dull	Agar: Wrinkled	Agar: Chromogenesis	Gel. Plate: Round, Compact	Gel. Plate: Proteus-like	Gel. Plate: Rhizoid	Gel. Plate: Filamentous	Gel. Plate: Curled	Gel. Stab: Funnel	Gel. Stab: Surface Growth	Gel. Stab: Needle Growth	Potato: Abundant	Potato: Discolored	Grows at 37° C	Liquefaction: Gelatin[3]	Liquefaction: Casein	Liquefaction: Blood Serum	Milk: Curdled	Milk: Acid	Milk: Alkaline	Indol[4]	H_2S.	Ammonia[4]	Nitrates Reduced[4]	
Cult. No.																																
Name																																
Source																																

DETAILED FEATURES.

I MORPHOLOGY.[2]

1. Vegetative Cells.

Form. *round, short rods, long rods, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate.*

Limits of Size.

Ends. *rounded, truncate, concave.*

Agar / Block: Orientation, (grouping). Chains (No. of elements)..... *short chains, long chains*. Orientation of Chains, *parallel, irregular.*

2. Sporangia.

Form, *elliptical, short rods, spindled, clavate.*

Limits of Size.

Agar / Block: Orientation (grouping). Chains (No. of elements). Orientation of Chains.

Location of Endospores.

3. Endospores

Form *round. elliptical, elongated.*

Limits of Size.

Wall.

Naked.

Sporangium wall adherent.

Germination, *equatorial oblique, polar, bipolar, by stretching.*

4 Flagella No.. .....

Arrangement.

5 Capsules, present on.....

6. Staining Reactions.

1.10 watery fuchsin.....

Methylene blue.

Special Stains.....

Fat.. .....

Neisser.....

Glycogen.....

II. CULTURAL FEATURES.

1. Agar Stroke.

Form of growth, *filiform, echinulate, beaded, spreading plumose, arborescent, rhizoid.*

Elevation of growth, *flat, effused, raised, convex.*

Lustre, *glistening, dull, cretaceous*

Topography, *smooth, contoured, rugose, verrucose.*

Optical Characters *opaque, opalescent.*

Chromogenesis.

Consistency, *slimy, butyrous, viscid, membranous coriaceous, brittle.*

Medium discolored.

2. Potato.

Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*

Elevation of growth, *flat, effused, raised, convex.*

Lustre, *glistening, dull, cretaceous.*

Topography, *smooth, contoured, rugose, verrucose.*

Chromogenesis.

Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*

Medium discolored.

3. Blood serum.

Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*

Elevation of growth, *flat, effused, raised, convex.*

Lustre, *glistening, dull, cretaceous.*

Topography, *smooth, contoured, rugose, verrucose.*

Chromogenesis.

Medium discolored.

Liquefaction.....10d.....4 w .....

4. Agar Stab.

Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*

5. Gelatin Stab.

Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*

Form of Liquefaction,[3] *crateriform, naviform, infundibuliform, saccate, stratiform.*

Surface growth.

6. Broth.

Surface growth, *ring, scum, floculent, membranous, none.*

Turbidity, *slight, moderate, strong, transient, persistent, none.*

7. Milk.

Coagulation.

Liquefaction .....10 d.....4 w .....

Reaction in 2 d.....4 d.....10 d .....

Consistency, *slimy, viscid, unchanged.*

Medium discolored.

Milk agar.

8. Gelatin Colonies.

Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*

Elevation, *flat, effused, raised, convex, pulvinate, crateriform, (liquefying).*

Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, flocose, curled.*

Internal structure, *amorphous, finely—coarsely-granular, grumose, filamentous, flocose, curled.*

9. Agar Colonies.

Form, *punctiform, round, irregular, ameboid mycelioid, filamentous, rhizoid.*

Elevation, *flat, effused, raised, convex, pulvinate, crateriform, (liquefying).*

Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, flocose, curled.*

Internal structure, *amorphous, finely,—coarsely-granular, grumose, filamentous, flocose, curled.*

10. Relative growth at 20° and 37°C.

III. BIOCHEMICAL FEATURES.

Fermentation of	Dextrose	Lactose	Saccharose		
Gas Production					
H. CO_2 ratio					
Growth in closed arm					
Acidifying Coefficient[5] 2d.					
" " 4d.					
" " 10d.					

2. Ammonia production,[4] *feeble, moderate, strong.*

3. Reduction of nitrates in nitrate broth.[4]

Presence of nitrites.....

" " ammonia.....

" " nitrates.....

" " free nitrogen .....

4. Starch Jelly.

IV. PATHOGENESIS.

Mice, Rats, Guinea Pigs, Rabbits, Man.

1. Toxin-forming.
2. Non-toxin forming.
3. Immunity bactericidal.
4. Immunity non-bactericidal.

V. ADDITIONAL DATA.

NOTE.—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

FIG. 9.— BACK OF SECOND SOCIETY CARD

GLOSSARY OF TERMS.

ARBORESCENT, a branched, tree-like growth.
AMEBOID, assuming various shapes like an ameba.
BEADED, in agar strokes, disjointed or confluent colonies; in agar stab, disjointed or confluent colonies along the line of inoculation.
BRITTLE, growth dry, friable under the platinum needle.
BULLATE, growth rising in coxvex prominences, like a blistered surface.
BUTYROUS, growth of a butter-like consistency.
CHAINS,
Short chains, composed of 2—8 elements.
Long chains composed of more than 8 elements.
CILIATE, having fine, hair-like extensions, like cilia.
CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.
CONVEX, surface the segment of a circle, but flatly convex.
CORIACEOUS, growth tough, not yielding to the platinum needle.
CRATERIFORM, round, depressed, due to the liquefaction of the medium.
CRETACEOUS, growth opaque and white, chalky.
CURLED, composed of parallel chains in strands, as in anthrax colonies.
ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.
EFFUSED, growth thin, veily, usually spreading.
ENTINE, smooth, having a margin destitute of teeth or notches.
EROSE, border irregularly toothed.
FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.
FILIFORM, in agar stroke a uniform streak along line of inoculation; in stab cultures a uniform growth along line of inoculation.
FIMBRIATE, border fringed with slender processes, larger than filaments.
FLOCOSE, growth composed of short curved chains, variously orientated.
INFUNDIBULIFORM, form of a funnel or inverted cone.
LACERATE, having the margin cut into irregular segments as if torn.
LOBATE, border deeply undulate, producing lobes (see undulate).
MEMBRANOUS, growth thin, coherent, like a membrane.
MYCELIOID, colonies having the radiately filamentous appearance of mold colonies
NAPIFORM, liquefaction with the form of a turnip.
PLUMOSE, a fleecy or feathery growth.
PULVINATE, in the form of a cushion, decidedly convex.
PUNCTIFORM, very minute colonies, whose form cannot be seen with the naked eye.
RAISED, growth thick, with abrupt or terraced edges.
RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides.*
RUGOSE, wrinkled.
SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.
SPREADING, growth extending much beyond the line of inoculation.
UNDULATE, border waved, with shallow sinuses.
VERRUCOSE, growth wart-like, with wart-like prominences.
VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils, as in potato cultures of the potato bacillus.
VILLOUS, growth beset with hair-like extensions.
VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

For further terminology see Manual of Determinative Bacteriology, Chester, 1901, pp. 381-86.

TABLE I.

A Numerical System of Recording the Salient Characters of an Organism.

100.	Endospores produced
200.	Endospores not produced.
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.0001	Nitrates reduced
.0002	Nitrates not reduced
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	"
.00000	Non-chromogenic

The genus according to the system of Migula is given its proper symbol which precedes the number thus:-

BACILLUS COLI (Escherich) Migula becomes B. 222.11110
BACILLUS ALCALIGENES Petruschky " " B. 212.33310
PSEUDOMONAS CAMPESTRIS (Pammel) Smith Ps. 211.33315
BACTERIUM SUICIDA Migula " Bact. 222.2320

NOTES.

(1) For decimal system of group numbers see Table 1.

(2) Observations on morphology of vegetative rods are to be made on 18 to 24 hour agar stroke cultures grown under optimum conditions. Observations of cultural features are to cover a period of at least 10 days. The composition, method of preparation, and reaction of all media should be given in connection with the description of cultural features (See revised methods of Laboratory Section of Am. Pub. Health Assoc., 1905).

(3) Gelatin stab cultures should be held for 4 weeks to determine liquefaction.

(4) Ammonia, indol and nitrite tests to be made at end of tenth day.

(5) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. Acidifying coefficient equals titre of culture divided by titre of blank.

The titration should be done after boiling to drive off any CO_2 present in the culture.

FIG. 10.— THIRD SOCIETY CARD; WITH RECORD OF CHARACTERISTICS OF *Ps. campestris*.

Source Composite Summary Date of Isolation ..... Name Ps. campestris (Pam.) Smith Group No. (1) Ps 211.3332513

DETAILED FEATURES.

NOTE—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

I. MORPHOLOGY (2)

1. Vegetative Cells, Medium used.....
temp.....age.....days
Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
Limits of Size.....
Size of Majority.....
Ends, *rounded, truncate, concave.*
Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements).... *Short chains, long chains* Orientation of Chains, *parallel, irregular.*
2. Sporangia, medium used.....temp.....age.....days
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of Size..... Size of Majority.....
Agar Hanging-Block: Orientation (grouping)..... Chains (No. of elements)..... Orientation of Chains, *parallel, irregular.*
Location of Endospores, *central, polar.*
3. Endospores.
Form, *round, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching.*
4. Flagella No.....Attachment *polar, bipolar, peritrichiate.* How Stained.....
5. Capsules, present on.....
6. Zoogloea, Pseudozoogloea.
7. Involution Forms, on.....in...days at...°C
8. Staining Reactions.
1:10 watery fuchsin, gentian violet, carbol fuchsin, Loeffler's alkaline methylene blue.
Special Stains
Gram.....Glycogen.....
Fat.....Acid fast.....
Neisser.....

II. CULTURAL FEATURES (3)

1. Agar Stroke.
Growth, *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*
Chromogenesis (6) Yellow.....
Odor, *absent, decided, resembling*.....
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*
2. Potato.
Growth *scanty, moderate, abundant, transient, persistent.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (6) Yellow..... Pigment in water *insoluble, soluble* other solvents.....
Odor, *absent, decided, resembling* NH_3.....
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*
3. Loeffler's Blood Serum.
Stroke *invisible, scanty, moderate, abundant.* Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (6).....
Medium *grayed, browned, reddened, blued, greened.*
Liquefaction begins in.....d, complete in.....d.
4. Agar Stab.
Growth *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, wide-spread.*
Line of puncture *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*
5. Gelatin Stab.
Growth *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
Liquefaction, *crateriform, naviform, infundibuliform, saccate, stratiform;* begins in 3-18..d, complete in.....d.
Medium *fluorescent, browned,*.....
6. Nutrient Broth.
Surface growth, *ring, pellicle, flocculent, membranous, none.*
Clouding *slight, moderate, strong: transient, persistent; none; fluid turbid.*
Odor, *absent, decided, resembling* Sweet corn
Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.*
7. Milk.
Clearing without coagulation.
Coagulation *prompt, delayed, absent.*
Extrusion of whey begins in 3-10 days.
Coagulum *slowly peptonized, rapidly peptonized.*
Peptonization begins on 3-10d, complete on 15..d
Reaction, 1d...., 2d...., 4d...., 10d...., 20d..N..
Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*
8. Litmus Milk.
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow reduction.
9. Gelatin Colonies.
Growth *slow, rapid.*
Form, *punctiform, round, irregular, ameboid, myceloid, filamentous, rhizoid.*
Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*
10. Agar Colonies.
Growth *slow, rapid,* (temperature 28 °C.)
Form, *punctiform, round, irregular, ameboid, myceloid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, radiate, striate.*
Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*
11. Starch Jelly.
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained.....
12. Silicate Jelly (Fermi's Solution.)
Growth *copious, scanty, absent.*
Medium stained.....
13. Cohn's Solution.
Growth *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*
14. Uschinsky's Solution.
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*
15. Sodium Chloride in Bouillon,
Per cent inhibiting growth.....
16. Growth in Bouillon over Chloroform, *unrestrained, feeble, absent.*
17. Nitrogen. Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*
18. Best media for long-continued growth.....
.....
19. Quick tests for differential purposes.....
.....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

1. Fermentation-Tubes containing peptone-water or Sugar-free bouillon and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit	Nitrate
Gas production, in per cent.							
$\left(\frac{H}{CO_2}\right)$							
Growth in closed arm							
Amount of acid produced 1d.							
" " " " 2d.							
" " " " 4d.							

IV. PATHOGENICITY.

1. Pathogenic to Animals
Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.....
2. Pathogenic to Plants: Cabbage.....
.....
.....
3. Toxins, *soluble, endotoxins.*
4. Non-toxin forming.
5. Immunity bactericidal.
6. Immunity non-bactericidal.
7. Loss of virulence on culture media: *prompt, gradual, not observed* in.....months.

Substance	Method used.	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY (2)	Diameter over 1 μ	O
	Chains, filaments	O
	Endospores	+
	Capsules	—
	Zoogloea, Pseudozoogloea	—
	Motile	+
	Involution forms	—
CULTURAL FEATURES (3)	Gram's Stain	+
	Broth: Cloudy, turbid	+
	Broth: Ring	+
	Broth: Pellicle	+
	Broth: Sediment	+
	Agar: Shining	O
	Agar: Dull	+
	Agar: Wrinkled	O
	Agar: Chromogenic	+
	Gel. Plate: Round	O
	Gel. Plate: Proteus-like	O
	Gel. Plate: Rhizoid	O
	Gel. Plate: Filamentous	O
	Gel. Plate: Curled	O
	Gel. Stab: Surface-growth	+
	Gel. Stab: Needle-growth	O
	Potato: Moderate, absent	+
	Potato: Abundant	+
	Potato: Discolored	+
	Potato: Starch destroyed	+
	Grows at 37° C.	+
	Grows in Cohn's Sol.	+
	Grows in Uschinsky's Sol	+
BIOCHEMICAL FEATURES	Liquefaction: Gelatin (4)	+
	Liquefaction: Blood-serum	+
	Liquefaction: Casein	+
	Liquefaction: Agar, mannan	—
	Milk: Acid curd	+
	Milk: Rennet curd	+
	Milk: Casein peptonized	+
	Indol (5)	—
	Hydrogen sulphide	—
	Ammonia (5)	+
	Nitrates reduced (5)	O
	Fluorescent	O
	Luminous	O
DISTRIBUTION	Animal pathogen, epizoon	+
	Plant pathogen, epiphyte	+
	Soil	—
	Milk	—
	Fresh water	—
	Salt water	—
	Sewage	—
	Iron bacterium	—
	Sulphur bacterium	—

FIG. 11.—BACK OF THIRD SOCIETY CARD.

DESCRIPTIVE CHART——SOCIETY OF AMERICAN BACTERIOLOGISTS.

Prepared by F. D. Chester, F. P. Gorham, Erwin F. Smith, Committee on Methods of Identification of Bacterial Species.

Endorsed by the Society for general use at the Annual Meeting, Dec. 31, 1907

GLOSSARY OF TERMS.

AGAR HANGING BLOCK, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID, assuming various shapes like an ameba.

AMORPHOUS, without visible differentiation in structure.

ARBORESCENT, a branched, tree-like growth.

BEADED, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF, a few days, a week.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS,
Short chains, composed of 2 to 8 elements.
Long chains, composed of more than 8 elements.

CILIATE, having fine, hair-like extensions, like cilia.

CLOUDY, said of fluid cultures which do not contain pseudozoogloeae.

COAGULATION, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flattened.

COPROPHYL, dung bacteria.

CORIACEOUS, growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

CURLED, composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION, Same as DIASTATIC, conversion of starch into water-soluble substances by diastase.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSE, growth thin, veily, unusually spreading.

ENTIRE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.

FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.

FILIFORM, in stroke or stab cultures a uniform growth along line of inoculation.

FIMBRIATE, border fringed with slender processes, larger than filaments.

FLOCCOSE, growth composed of short curved chains, variouly oriented.

FLOCCULENT, said of fluids which contain pseudozoogloeae, i. e., small adherent masses of bacteria of various shapes and floating in the culture fluid.

FLUORESCENT, having one color by transmitted light and another by reflected light.

GRAM'S STAIN, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismark brown.

GRUMOSE, clotted.

INFUNDIBULIFORM, form of a funnel or inverted cone.

IRIDESCENT, like mother-of-pearl. The effect of very thin films.

LACERATE, having the margin cut into irregular segments as if torn.

LOBATE, border deeply undulate, producing lobes (see undulate.)

LONG, many weeks, or months.

MAXIMUM TEMPERATURE, temperature above which growth does not take place.

MEDIUM, several weeks.

MEMBRANOUS, growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE, temperature below which growth does not take place.

MYCELIOID, colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM, liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS, the necessary nitrogenous food This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

OPALESCENT, resembling the color of an opal.

OPTIMUM TEMPERATURE, temperature at which growth is most rapid.

PELLICLE, in fluid bacterial growth either forming a continous or an interrupted sheet over the fluid.

PEPTONIZED, said of curds dissolved by trypsin.

PERSISTENT, many weeks, or months.

PLUMOSE, a fleecy or feathery growth.

PSEUDOZOOGLOEAE, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloeae.

PULVINATE, in the form of a cushion, decidedly convex.

PUNCTIFORM, very minute colonies, at the limit of natural vision.

RAISED, growth thick, with abrupt or terraced edges.

RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides*.

RING, Same as RIM, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

REPAND, wrinkled.

RAPID, Developing in 24 to 48 hours.

SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

SLOW, requiring 5 or 6 days or more for development.

SHORT, applied to time, a few days, a week.

SPORANGIA, cells containing endospores.

SPREADING, growth extending much beyond the line of inoculation, i. e., several millimeters or more.

STRATIFORM, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.

THERMAL DEATH-POINT, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT, a few days.

TURBID, cloudy with flocculent particles; cloudy plus flocculence.

UMBONATE, having a button-like, raised center.

UNDULATE, border wavy, with shallow sinuses.

VERRUCOSE, growth wart-like, with wart-like prominences.

VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils.

VILLOUS, growth beset with hair-like extensions.

VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

SOOGLOEAE, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenterioides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering, inside of which there may be one or many groups of the bacteria.

NOTES.

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth.) Growths at 37° C shall be in general not older than 24 to 48 hours, and growths at 20°C not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practiced as described in the revised Report af the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for 6 weeks to determine liquefaction

(5) Ammonia and indol tests shall be made at end of 10th day, nitrite tests at end of 5th day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

The titration should be done after boiling to drive off any CO_2 present in the culture.

(7) Generic nomenclature shall begin with the year 1872 (Cohn's first important paper.)

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms.)

(8) Chromogensis shall be recorded in standard color terms.

TABLE I.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM. (GROUP NUMBER)

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
00001	Fluorescent
.00002	Violet chromogens
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerine
.0000002	Acid without gas from glycerine
.0000003	No acid from glycerine
.0000004	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig.	becomes	B.	222.111102
BACILLUS ALCALIGENES Petr.	"	B.	212.333102
PSEUDOMONAS CAMPESTRIS (Pam.) Sm.	"	Ps.	211.333151
BACTERIUM SUICIDA Mig.	"	Bact.	222.232203

and glossary are more extensive. Under brief characterization the record is to be made by + and 0 instead of + and —. The card now provides for all of the observations which are commonly made in connection with bacteria and many of the unusual ones.

It is evident that the present card system of classification is the result of a steady and logical growth and the names of Johnson, Fuller, Conn, Gage, Chester and Smith mark important stages in its progress. While each of them added something of their own they brought much more from the experience of their associates, building gradually the foundations of the science of bacteriology.

CONSTANCY OF THE BASIS FOR THE GROUP NUMBER.

While the group number, brief characterization and detailed description, as previously described, are all important, the maximum weight attaches to the group number, since this is the basis upon which the collections of cards are arranged. Any variation in this number, in the case of strains of the same species, will separate the representatives of this species.

As already stated the species selected for this study was *Pseudomonas campestris* (Pam.) Smith, a well-known, chromogenic, plant pathogen; since with this species the question of contamination could be easily settled.

Variations in bacteria are sometimes ascribed to the differences in climate and food in nature. In order to observe the result of varied climatic environments cultures were kindly provided by Prof. S. A. Beach from Iowa, Prof. W. J. Morse from Maine, Prof. F. L. Stevens from North Carolina and Dr. Erwin F. Smith from Washington, D. C., in addition to a large number of cultures isolated from widely separated points in this State.

When the group number was determined in accordance with the directions prepared by the Society of American Bacteriologists the group number 211.3332513 was identical for all of these cultures.

Although difference in natural environment between points separated by more than a thousand miles was evidently not sufficient to affect these physiological reactions of this species it is conceivable that the vicissitudes of moisture and of food to which artificial cultures are exposed might be more potent in this connection. Accordingly comparisons were made between the group number of cultures freshly isolated from diseased cabbage and that of cultures which had been long in cultivation in this and other laboratories. This observation of the effect of cultivation in this laboratory covered sixteen months. Some of the cultures had, at the beginning of this period, been cultivated for months if not years, while others had been freshly isolated from the diseased plants.

The group numbers obtained for these various strains, with certain exceptions to be later discussed, were identical, strongly indicating that artificial cultivation does not induce changes in the group number of this species.

In presenting the results of this study the fact that there were some apparently discordant observations should not be overlooked. It is highly probable that the common conception of the variability of bacteria is largely due to such discordant observations which have not been traced to their true causes.

In shipping cultures through the mail where they are exposed to the curiosity of the mail clerks, in the exposure accompanying the repeated transfer of stock cultures and in selecting typical colonies as a basis for stock cultures from plates, either in isolation from diseased plants or in the revivifying process, there is a constant opportunity for contamination and mistakes in judgment. Only the most careful attention to details will detect all of these contaminations.

In this study, including over 50 strains, there were five cases where the group number was determined for cultures which had the gross appearance of *Ps. campestris* in colony growths but a detailed study showed that they were not this organism.

There were two additional cases of discordant observations which could not be so easily explained. A culture of our own isolation, strain 3, after being cultivated in the laboratory for eight months produced a faint acidity in fermentation tubes containing respectively lactose, saccharose and glycerin. Two months later this same strain, tested on cabbage in the green-house, produced the typical black rot. A culture reisolated from one of these diseased plants, strain 80, did not give the above anomalous acid reaction with lactose, saccharose and glycerin but gave the typical group number. It was not possible to determine more closely the cause of these unusual reactions with strain 3 since it had been accidentally lost. This may have been a true case of variation but the failure of the acquired fermentative ability to persist in the culture reisolated from the diseased plant would suggest that the original group number determination was confused by the presence of an undetected acid-forming contamination which was excluded by passing the culture through the cabbage plant.

Acid formation was also observed with strain 65, which was isolated from diseased cabbage from Long Island. After being cultivated two months in the laboratory it was inoculated into cabbage in the green-house and gave no outward evidence of pathogenicity. On dissecting the plants a few blackened bundles were found close to the point of inoculation in one plant and reisolations from these bundles gave strain 98. A determination of the group number of each strain now showed the formation of acid in the presence of dextrose, lactose, saccharose and glycerin with each, a reaction which is not typical of *Ps. campestris.*

Immediately after these determinations strain 98 was inoculated into three cabbage plants in the field with the usual precautions, the inoculation being made through the base of a severed leaf, the point of inoculation being carefully covered with sterile, melted, grafting wax and suitable check inoculations being made on adjoining plants at the same time. Each of three plants thus

inoculated with strain 98 developed a well-marked case of black rot while the adjoining check plants remained healthy. The disease began at the point of inoculation and was traced by the blackened fibro-vascular bundles into a large number of the leaves. Cultures made from these blackened bundles, several inches from the original point of inoculation, gave an apparently pure culture, strain 100. The group number of this strain was typical, there being no evidence of acid formation from any of the sugars or from glycerin.

From a superficial observation of these results with strains 65, 98 and 100 one might conclude that here was an acquisition and later a loss of the ability to form acid, corroborating the observations with strains 3 and 80. This view is strengthened by the retention of this acid-forming character after passage through one cabbage plant even though this ability was lost in passing through the second plant.

A careful study of these observations shows that the above idea has really little basis in fact and that these apparently discordant observations were probably due to unrecognized contaminations.

The strongest evidence against the idea of contaminated cultures is the appearance of this acid-formation in two strains, 65 and 98, the latter after the passage of the culture through a cabbage plant. However, in this case there were no lesions produced except a blackening of the fibro-vascular bundles at the point of inoculation and the material for strain 98 was necessarily taken from practically the point where strain 65 was inoculated. Russell[25] showed that even ordinary saprophytes are able to survive at the point of inoculation in plants for some months so that in this case the survival of a mixed culture is neither impossible nor improbable. On the other hand when strain 98 was inoculated into cabbage it produced an extensive disease so that the material from which strain 100 was derived came from a distant portion of the plant and the contamination present in the inoculating material was thus mechanically removed.

[25] Russell, H. L. Bacteria in their relation to vegetable tissue. From Johns Hopkins Hospital Reports, 3: 223–263. 1893.

If we consider more closely the fermentation tube tests of strains 3 and 80 and strains 65, 98 and 100 it will be noticed that strain 3 did not form acid from dextrose while strains 65 and 98 formed acid from this sugar. Also the formation of acid from various substances by strain 3 was not accompanied by any growth in the closed arm while with both strains 65 and 98 this growth was abundant under such circumstances. Thus if the ability to form acid is to be considered as an acquired characteristic of strains 3 and 65 it is evident that in the two instances this ability was acquired with regard to different sugars and further in the latter case the relation to oxygen was also profoundly modified. In strain 100, both of these modifications had been lost.

Fortunately strains 65 and 98 were available for study although seven months had elapsed since the previous test. At this time strain 65 was less chromogenic although it gave the reactions with fermentation tube tests which are recorded for the original strain 65. On the other hand strain 98 was typically chromogenic, did not grow in the closed arm of the fermentation tubes and did not form acid from the sugars or glycerin. The repeated transfers of the stock cultures had resulted in a pure culture of the contamination, which was a bacillus with the group number B. 211.2223532, on one hand and of the true *Ps. campestris* on the other.

The observations on nitrate reduction were the occasion of some confusion during the earlier determinations. Where the cultures were tested for the presence of nitrites by the official sulphanilic acid method there was at times a faint reaction for nitrites which seemed more marked than the reaction obtained from the control tubes. This method of detecting nitrites is so delicate that the nitrites ordinarily absorbed by the control tubes give a faint reaction. When a large number of control tubes were tested, the intensity of the color was not the same in all of the tubes, even when originally filled from the same flask of media. Accordingly when testing a large number of inoculated tubes with

a small number of controls it was probable that some of the inoculated tubes would give a stronger reaction than the controls.

A large number of cultures were prepared, using all of the strains where nitrate reduction had been previously recorded, and comparative tests were made, after the proper interval, by the official sulphanilic acid and by the KI–H_2SO_4– starch[26] methods. In these tests both the control tubes and a number of the inoculated ones gave irregular results by the official method but in no case was there the slightest evidence of nitrite formation by the KI–H_2SO_4– starch method. Although the sulphanilic acid method is official it is evident that it is too delicate for satisfactory results where media are kept for a number of days in an ordinary laboratory.

It will be observed that this failure to reduce nitrates is not in agreement with the reaction indicated by the illustrative group number of *Ps. campestris* as given on the official card. However Dr. Erwin F. Smith[27] states that this number on the card is a typographical error and not in accord on this point with any previous data.

Varying results are to be expected in the test for the diastatic action of the organism on potato starch, since the official methods do not set time limits nor prescribe methods for making the test. In this study the cultures were made on potato cultures in large test tubes, containing a supply of water and the starch test applied after two weeks. For this purpose an alcoholic solution of KI–I was added separately to the water in the culture tube and to the potato mass after crushing in water. A faint action on the starch is shown by a wine color in the water in the culture tube where such action would pass unnoticed with the potato mass. Naturally the extent of the diastatic action turns both on the age and vigor of the culture used. The action was always well marked and often practically complete after two weeks.

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[26] The details of this method are given by Smith, E. F. Bacteria in relation to plant diseases. Vol. I, p. 63. 1905.

[27] In a personal letter.

CONSTANCY OF "BRIEF CHARACTERIZATION" ITEMS.

The present application of the Society Card to classification is primarily in connection with the group number. However, as has been pointed out by Bergey and Bates,[28] it will undoubtedly be necessary to extend this number to additional reactions before the group number will separate bacteria down to the groups which we ordinarily designate as species. In searching for suitable reactions to be used in this connection attention turns first to those items included under "Brief Characterization." Constancy in the results of tests with different strains of the same species is the important point in this connection and accordingly observations on many of the headings given on the card under brief characterization were made when the group number was being determined.

The results of these observations are given in Table I.

[28] Bergey, D. H. and Bates, H. L. The numerical classification of bacteria. From the Univ. of Pa. Medical Bulletin. July, 1906.

TABLE I.—ACTION OF STRAINS OF P. CAMPESTRIS WITH RESPECT TO "BRIEF CHARACTERIZATION" ITEMS.

Source:	No. of Strain	Months in Lab.	Plant pathogen, epiphyte	Milk		Liquefaction			Potato			Gel. Stab	Gel. Plate.			Agar		Broth		
				Indol (?)	Casein peptonized	Rennet curd	Acid curd	Agar	Casein	Gelatin (4)	Grows in Uschinsky's Sol.	Grows in Cohn's Sol.	Starch destroyed	Discolored	Abundant	Moderate	Needle-growth	Surface-growth	Curled	Filamentous	Rhizoid	Proteus-like	Round	Chromogenic	Wrinkled	Dull	Shining	Sediment	Pellicle	Ring	Cloudy, turbid
Geneva	3	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
Iowa	4	7	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
Geneva	6	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	8	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	12	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	13	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	13	14	+	.	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	14	8	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	15	11	+	0	+	+	+	0	+	+	+	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	16	8	+	0	.	.	.	0	+	.	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	16	14	+	.	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
Long Island	19	10	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	19	16	+	.	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	20	15	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	25	12	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
Maine	31	1	.	0	+	+	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	31	3	0	0	+	0	0	0	+	+	0	0	+	+	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
Washington	34	4	+	0	+	.	.	0	+	+	+	+	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
Geneva	35	4	+	0	+	.	.	0	+	+	+	+	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	39	.	.	0	+	.	.	0	+	+	+	+	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	40	.	.	0	+	0	0	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	40	.	0	0	+	0	0	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
North Carolina	45	.	.	0	+	0	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
Long Island	50	0	+	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	50	7	.	.	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	51	0	+	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	52	7	.	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	52	0	.	+	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	53	0	.	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	53	7	.	.	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	54	0	.	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	54	7	.	.	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
"	55	3	+	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	60	3	+	0	+	+	0	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
"	65	3	+	+	+	+	+	0	+	+	0	0	+	.	+	0	+	+	.	.	.	.	+	+	0	0	+	0	0	0	+
No. 3	80	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 4	81	0	0	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 6	82	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 8	83	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 12	84	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 13	85	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 14	86	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 15	87	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 16	88	0	+	0	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 19	89	0	+	0	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 50	90	0	+	0	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 32	92	0	+	+	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 34	93	0	+	0	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 35	94	0	+	0	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 55	96	0	+	+	+	0	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 60	97	0	+	+	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 65	98	0	+	+	+	+	+	0	+	+	0	0	+	.	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+
" 98	100	3	.	.	+	+	+	0	+	+	0	0	+	0	+	0	+	+	0	0	0	0	+	+	0	0	+	0	0	0	+

These results are arranged in the order of the serial numbers of the strains, a positive reaction being indicated by +, a negative by 0 and the lack of data by . . .

Months in the laboratory.— It will be noted that determinations of strains 50–54 and 80–98 were made promptly after they had been isolated from diseased material. Accordingly these determinations represent *Ps. campestris* when showing the minimum effect of artificial cultivation. The maximum effect of such cultivation here shown is sixteen months in the case of strain 19. Aside from the cultures which were obtained from other laboratories and concerning the previous history of which little is known, the numerals in this column indicate the period during which the strains had been cultivated previous to having their characteristics determined. A second determination after an interval was made in the case of strains 13, 16, 19, 31, 40, 50, 52, 53 and 54.

Pathogenicity.— This was determined by inoculations from forty-eight-hour agar slopes into young, rapidly growing cabbage plants in the green-house, inoculating through the base of a severed leaf by means of a sterilized platinum needle dipped in the culture, the inoculation being covered with sterile melted grafting wax. Similar check inoculations were made at the same time using the same sterile platinum needle and other untreated plants were also retained in connected with the inoculated plants in the green-house. The first evidence of disease usually appeared in about ten days, showing as blackened veinlets in the lamina of one or more leaves above the point of inoculation. In all of these tests no disease appeared in any of the check plants or in the untreated controls. There is a common impression that the pathogenicity of bacteria toward plants is an extremely variable matter. These results show only three failures of pathogenicity to manifest itself out of forty-one tests, notwithstanding the fact that a large part of these strains had been cultivated eight months or more before being tested. On the other hand it is true that the degree of pathogenicity is somewhat variable. Inoculations

were made in triplicate on the same day, using cabbage plants which had grown from seed sown on the same day, inoculating from agar slopes of the same age and composition. With some strains disease was evident in all three plants in ten days while others presented lesions only after three weeks. The basis of this variation is not yet recognized but probably lies in faulty technique, the cultures not being brought to standard conditions in some particular before the inoculations are made.

Indol.— Five strains are recorded as positive at a single examination. Strain 52 was negative seven months later and evidence has already been presented to show that strains 65 and 98 were contaminated when tested. The indol formation has not been redetermined for strains 92 and 96. In no case was there evidence of strong indol formation but with a number of other strains single tubes in the triplicate test gave faint indications of indol formation. The tests were made at the end of ten days by adding two drops of concentrated sulphuric acid and 1 c.c. of a 0.01 per ct. solution of sodium nitrite to the culture in 1 per ct. peptone water, taking the color reading at the end of thirty minutes.

These results would indicate that the tendency to indol formation is stronger in freshly isolated cultures and that a careful study of the causes of this variation should precede the adoption of indol formation as a basis of classification.

Casein peptonized.— The results are all positive since *Ps. campestris* is an active digester. The lack of a simple and accurate test of the beginning of digestion limits the usefulness of this reaction with many organisms. A search for such a test should include a consideration of both Hasting's[29] work and a chemical determination of the digestion products either with or without dialysis.

Rennet and acid curd.— Milk was frequently digested by this species without any apparent curdling. As acid was absent, except in a single instance, the curdling which occurred was at-

[29] Hastings, E. G. The action of various classes of bacteria on casein as shown by milk-agar plates. *Cent. f. Bak.* **II, 12**: 590–592. 1904.

tributed to rennet. The distinction between rennet and acid curds is not sufficiently clear cut to be of much value in classification.

Liquefaction.— The liquefaction of agar, while not unknown, is so rare as to be of little use as a distinguishing characteristic. Liquefaction of casein has been already discussed. Gelatin was uniformly liquefied but the rate of action varied widely. This action is recorded in the group number.

Uschinsky's solution.— The solution used had the following composition:

30.0 grams	glycerin	$C_3H_5(OH)_3$
5.0 "	sodium chloride	Na Cl.
0.1 "	calcium chloride	$CaCl_2$
0.3 "	magnesium sulphate	$MgSO_4$
6.0 "	ammonium lactate	$(NH_4)\ C_3H_5O_3$
2.0 "	di-potassium phosphate	K_2HPO_4
3.0 "	sodium asparaginate	$NaC_4H_6O_4$
1000 cc.	distilled water	

Growth was evident with approximately one-half of the strains and slight multiplication undoubtedly occurred with at least a part of the remainder.

Cohn's solution.— This solution had the following composition:

5.0 grams	acid potassium phosphate	KH_2PO_4
5.0 "	magnesium sulphate	$MgSO_4$
10.0 "	neutral ammonium tartrate	$(NH_4)_2C_4H_4O_6$
0.5 "	potassium chloride	KCl
1000 cc.	distilled water	

This solution was much less acceptable to *Ps. campestris* than the Uschinsky's solution, growth being evident with only 11 strains. When this determination was repeated with strains 31 and 40 the growth did not appear showing that even with the same strain the results were quite variable.

This marked irregularity in growth in these two solutions of known composition does not favor the idea that the use of syn-

thetic media is the true basis for exact comparative work. There seems no escape from the conclusion that there was some unrecognized factor which produced this variation and it is quite possible that were this recognized constant results might have been obtained.

Potato.— The starch was regularly attacked as described under group number. Beyond the limit of the visible growth the surface of the potato was rendered gray. At one stage of the culture a white margin or halo was formed just outside of the line of growth and this is indicated in the table by +. This was probably present in all cases but as this was a transient phenomenon it was not always recorded. The growth appeared quickly at 20–30°C. and soon became abundant in all but strain 98. Ordinarily the growth flowed from the potato to the bottom of the container within ten days.

Gelatin stab.—Although this species is so strong an aerobe that growth does not appear in the closed arm of the fermentation tube it uniformly takes place along the needle track. Surface growth is also always present. These two reactions, needle-growth and surface-growth, are of little value for classification not because they are variable but rather because practically all species agree in these particulars.

Gelatin plate.— In this division all of the strains agree in that the colonies are round. While other forms of colonies occur with a few species such species are very unusual. Moreover form of colony is so largely dependent on environment that it has little value for classification.

Agar.— The results under this head are entirely accordant, the cultures being yellow and shining. The color is a part of the group number. While no confusion would arise with this species from the use of the surface appearance for classification other species would undoubtedly be found where the record on this point would depend largely upon the personal equation.

Broth.— This species develops a finely granular turbidity and later forms a sediment but the turbidity was not noted with

strains 6 and 97 and the sediment was lacking with strains 84 and 100. Ring and pellicle formation were very irregular, each appearing with about one-half of the strains.

These results would indicate that data of value for classification might be obtained from pathogenicity to plants, indol formation, casein digestion, growth in Uschinsky and Cohn solutions and turbidity in broth. In all cases further study both of the technique of making tests and of the reactions of different species will be necessary before the relative constancy of these reactions can be settled.

CONCLUSIONS.

The present Classification Card of the Society of American Bacteriologists is the logical outcome of forces which have been at work among bacteriologists for at least fifteen years.

This card offers a basis for classification which in convenience of application and in certainty of result surpasses anything which has preceded it. In furnishing a form for recording and organizing a mass of observations it solves a difficulty which has frequently been the limiting factor in research. Intelligently applied it is calculated to bring order out of chaos and thereby become a most potent factor in raising bacteriology to the dignity of a true science.

When tested upon forty-four strains of *Pseudomonas campestris* (Pam.) Smith, the group number, as now constituted, gave constant results and did not break the species into smaller groups. Therefore so far as it applies to this species it is a satisfactory basis for classification. This study did not test the possibility of other species having this same group number.

The qualitative variations and apparently discordant reactions which have commonly been attributed to bacteria are probably due largely to faults either in observation or in technique. Quantitative variations are constantly met but these in turn are undoubtedly largely due to lack of knowledge concerning the proper revivifying process to be applied before determining the culture characteristics.